Synthesis of Zinc Oxide Nanoparticles Using *Ocimum Lamiifolium* Leaves Extract and its Antibacterial Activity

Yohannes Misskire[1]*, *Yemisrach Negalign*[1]

[1]Department of Chemistry, College of Natural and Computational Sciences, Debre Markos University, Debre Markos, Ethiopia

*Corresponding author Email: yohannes_miskir@dmu.edu.et ; yohasmw@gmail.com

Abstract

Green synthesis of nanoparticles using microorganisms and plant extracts has emerged as a new, eco-friendly and cost effective method. This study aimed at synthesizing zinc oxide nanoparticles (ZnO NPs) from Zn $(NO_3)_2$.$6H_2O$ solution and Ocimium lamiifolium (Damakessie) leaves extract under optimum conditions. The presence of phytochemicals in the leaves extract was identified using qualitative screening methods. The synthesized ZnO NPs were characterized by UV – Vis, FTIR and XRD spectroscopic techniques. Antimicrobial activity of the ZnO NP was evaluated by the disc diffusion method. The result was that the surface Plasmon resonance (SPR) in the UV –Vis spectroscopy indicated the reduction of the Zinc Oxide nanoparticles at 321 nm, which shifted from 361 nm in the plant extract. The FTIR analysis showed that the major functional groups in the leaves extract had an absorption band that shifted in the ZnO NPs. The X-ray diffraction pattern revealed the average particle size of ZnO powder at about 22.6 nm using the line width of the plane refraction peak. The ZnO NPs exhibited antimicrobial activities against Gram-positive and Gram negative bacteria with maximum zone of inhibition against E.coli (17.5 mm) whilst the least activity was against the S. aureus bacteria.

Keywords: *Nanoparticles; Ocimum-Lamiifolium; antibacterial activity; characterization techniques; ecofriendly*

Introduction

In many areas of current technologies, metal oxides with nanostructure attracted the attention of researchers [1]. Zinc oxide (ZnO) is a unique material that has received much attention from researchers of various disciplines such as in biomedical fields [2], semiconducting, piezoelectric, and pyroelectric properties, transparent electronics, ultraviolet (UV) light emitters, piezoelectric devices, chemical sensors, spin electronics, personal care products, coating and paints [3, 4], UV light emitting devices,

solar cells, photo catalysts, and gas sensors, cosmetic and pharmaceutical industries [5].

The synthesis of nanoparticles can be performed using a number of routinely used chemical and physical methods. However, these methods are energy and capital intensive, and they employ toxic chemicals and non- polar solvents in the synthesis procedure. Therefore, the need for the development of clean, reliable, biocompatible and ecofriendly processes grew up [6]. Green synthesis (biosynthesis) methods provide advancement over chemical and physical methods as it is cost-effective, environmentally friendly, easily scaled up for large scale synthesis and there is no need for high pressure, energy, temperature and toxic chemicals [7].

Plants are the major source of phytochemicals involved in the synthesis of stable nanoparticles for large-scale production [8]. Many different compounds are found in *O.lamiifolium* including alkaloids, terpenoids, flavonoids, steroids, glycosides, tannins, amino acids, vitamins and minerals [9]. It is one of the important generations of family *Lamiaceae,* which is often referred to as the “king’’ of the herbs. The plant is widely distributed in tropical and warm temperate regions of the world, especially in tropical America, Africa and Asia. It is also one of the most widely used medicinal plants in the Ethiopian traditional medicine. For instance, it is used for the treatment of inflammatory conditions and infections; the juice as an eye rinse to treat eye infections; the crushed leaves are put in the nostrils where they can stop nose bleeding [10].

Figure 1 The upper part of ocimiumlamiifolium (*Dama kessie*)

Ocimum Lamiifolium is widely used as a traditional medicinal plant in Ethiopia. Leaves of the plants are used to treat many diseases. The use of ocimum lamiifolium plant leaves for the synthesis of ZnO NPs was used because it is rapid, environmentally friendly, no pathogens are used and the whole process involves a single-step. So far, there have not been any reports on the synthesis of ZnO nanoparticles from the leaves of this plant extract as reducing, stabilizing, and capping agent. Therefore, this research was initiated to taste the phytochemicals present in the leaves of Ocimum lamiifolium, synthesize zinc oxide from zinc nitrate and leaf extracts of Ocimum lamiifolium, and evaluate the antibacterial activity of synthesized zinc oxide nanoparticles against gram-positive and gram-negative bacteria.

Materials and Methods

Chemicals and Reagents

The following chemicals and reagents were used for the study. A 0.1M Zn $(NO_3)_2.6H_2O$ solution, $FeCl_3$ (99%), HCl (35.4%), H_2SO_4 (98%) (Products of Loba Chemie Pvt.Ltd, India), C_2H_5OH (99.5%, UNI-CHEM Chemical Reagents),CH_3OH, Gentamicin (Abcekadtek (P) Ltd), KBr (Uvasol, Germany) Agar Hilten Muller (Oxoid CM, UK), Chloroform (99.9%, Fisher Scientific UK Limited, UK), NaOH (98%), NH_3 solution (25%) (Both of them are products of Blulux Laboratories (P) Ltd), Benedict's 8%), Iodine solution,(80%), KI (99%) (produced by Abron Chemicals, India). All

these chemicals and reagents were of analytical grade and used without any further purification.

Instruments

Among the instruments that were used for characterization of ZnO NPs include: UV-Vis, X-Ray Diffraction (XRD) (Agilent Technologies, Cary 60UV-Vis), Fourier Transform Infrared spectroscopy (FTIR) (Perkin Elmer) and other light instruments in the laboratory.

Experimental Methods

Collection and Preparation of the Plant Material

The synthesis of the nanoparticles used the leaves extract of a medicinal plant from Yemeka forest, near Debre Markos town, East Gojjam zone, North West Ethiopia. About ten leaves of the plant O. lamiifolium were collected and transported to Debre Markos University Chemistry Laboratory. Fresh O.lamiifolium leaves were washed with distilled water several times and dried for seven days in air and grounded by mortar. The extraction was done by taking 3 grams of the leaves powder followed by addition of 100 ml distilled water as a solvent in a 250 ml glass beaker. Then, it was boiled at 60 °C for 15 minutes. The final extract of the solution was collected and stored at 4 °C within a refrigerator. The extract was cooled to room temperature and filtered using filter paper and stored in a refrigerator for further experiment.

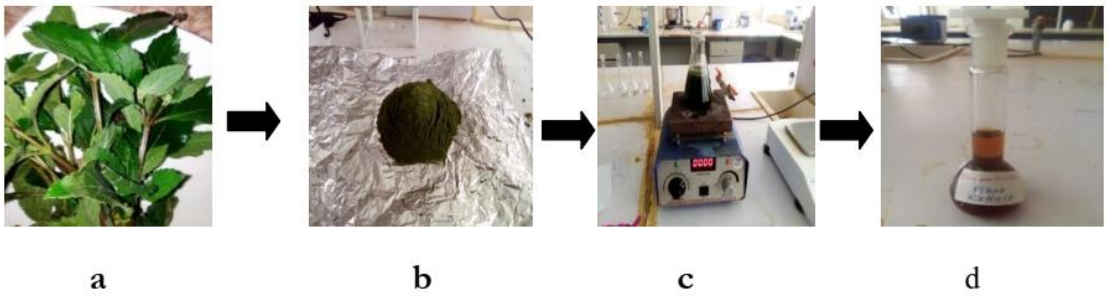


Figure 2. The general process for the preparation of O.lamiifolium leaves aqueous extract (a) The collected and washed O.lamiifolium leaves sample (b) the dried and crushed leaves in a foil paper (c) O.lamiifolium leaves were mixed with distilled water & heated (d) The solution was filtered & clear extracted solution was collected.

Phytochemical Screening of Leaf Extracts

The plant Ocimum Lamiifolium contains many different compounds: alkaloids, glycosides, tannins, flavonoids, terpenoids, amino acids, vitamins, and minerals [18]. The screening and identification of bioactive

chemical constituents like alkaloids, carbohydrates, glycosides, saponins was tested according to the following standard methods [15].

a. Test for Alkaloids (Wagner's test: I_2-KI solution)

b. Test for Glycosides (Alkaline reagent)

c. Test for Tannins (Ferric chloride test)

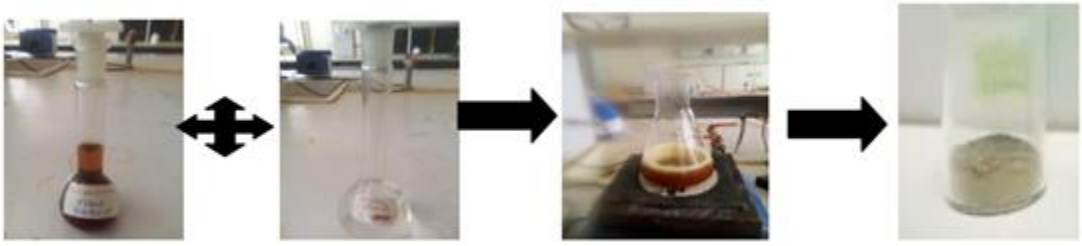
d. Test for Flavonoids (Ferric Chloride test)

e. Test for Steroids (Salkowski test)

f. Test for Phenols – $FeCl_3$ solution

Synthesis of ZnO nanoparticles

For the synthesis of ZnO nanoparticles (Figure 3), 15 ml of the leaves extract of Ocimum Lamiifolium plant was taken and boiled at 60 ^oC in a magnetic stirrer, followed by the addition of 30 ml of 0.1 M zinc nitrate solution onto it. The mixture was then boiled until it changed to yellow color paste that indicates the reduction of Zn and formation of its nanoparticles. Then, this paste was collected using aluminum foil and heated in the furnace at 400 ^oC for 2 hrs. The product obtained was gray in color and it was crushed into small powder by using a ceramic crucible. The powdered materials (nanoparticles) were put in the sample holder and packed, for next characterization.



(a) Extract (b) Precursor (c) Heating and Steering (d) ZnO NPs (powder)

Figure 3. Bio-synthesis processes of ZnO NPs

Antimicrobial Activity of ZnO NPs

Disc diffusion method

The disc diffusion method was employed for the antibacterial test due to its simplicity, low cost, versatility and the ease of interpretation [19]. A well-known antibiotic, gentamicin, was used as a control. Distilled water, O.lamiifolium leavers extract, ZnO nano particles and gentamycin in different beakers were prepared and emulsified in each colony in the above prepared solutions.

Results and Discussion

Visual observation

In the reaction mixture of leaves extract of O.lamiifolium and 0.1M $Zn(NO_3)_2$ solution, color change was observed from brown to yellow that may confirm the bio reduction of zinc ions to ZnO nanoparticles(Figure 4).

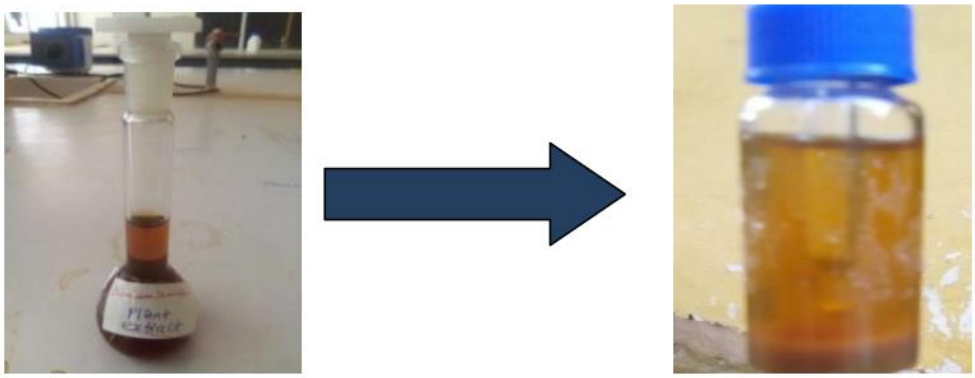


Figure 4. Color change as a result of the reaction between 0.1 M Zn $(NO_3)_2$ and plant leaves extract of O.lamiifolium plant

Phytochemical analysis

The results of the qualitative screening of phytochemicals in the leaves extract of O.lamiifolium are shown in Table 1 and Figure 5. It contained the alkaloid and other polyphenolic compounds such as tannins and flavonoids [15]. Therefore; O.lamiifolium leaves extract is composed of phytochemicals that are capable of reducing the Zn^{2+} by donating electrons, capping and stabilizing the formed nanoparticles. For instance, the polyphenolic compounds that are found in plants including O.lamiifolium are very important plant constituents because of the hydrogen abstraction due to the OH groups in the reductant molecules. The antioxidant property of polyphenolic compounds is mainly due to its redox property which allows them to act as reducing agents [15].

Figure 5. The colors observed when the extracted is tested for: (A) Alkaloids (B) Glycosides (C) Flavonoids (D) Steroid (E) Tannins and (F) Phenols

The color changes are indicators of the formation of different complexes as a result of oxidation and reduction reactions. For instance, the yellow color for alkaloids indicates that the nitrogen and/or oxygen atoms of the amide groups of the alkaloids involve in the chemical reaction (oxidation and reduction). In most of the ferric chloride tests, iron (III) forms complexes having different colors depending on the nature of the complexes [15].

Table 1. The qualitative analysis of phytochemicals in the Ocimum Lamiifolium plant leaves extract**.**

No.	Phytochemical	Chemical test	Results	Colors observed
1	Alkaloid	Wagner's test	++	Yellowish
2	Glycoside	Alkaline test	++	Reddish brown
3	Flavonoids	Ferric chloride test	++	Brown ppt
4	Steroid	Salkowski test	++	Brown Yellow
5	Tennis	Ferric chloride test	+	Yellow
6	Phenol	Ferric chloride test	++	Bluish black

Key: + indicates presence, ++ indicates highly presence

UV-Vis analysis

To examine the optical properties of nanoparticles, UV-Vis spectroscopic analysis was employed. In Figure 6, the distinct peak centered at 321 nm for ZnO NPs might be due to their large excitation binding energy at room temperature. When the band gap increases the particle size decreases. The band gap energy of ZnO NPs calculated by using the formula $E = hc/\lambda$ was found to be 3.8 ev and also from this peak, it can be inferred that nanoparticles are uniformly distributed and particles are nano sized. As there is no other peak observed in the whole spectrum, it implies that ZnO has been successfully formed [16].

The UV-Vis spectra of plant extract O.lamiifolium, the appearance of one or more peaks in the region from 200-400 nm is a clear identification of the presence of unsaturated groups and hetro atoms (S,N,O). The O.lamiifolium leaf extract shows one peak at position of 361 nm may confirm the presence of organic chromophores with in the plant extract [16].

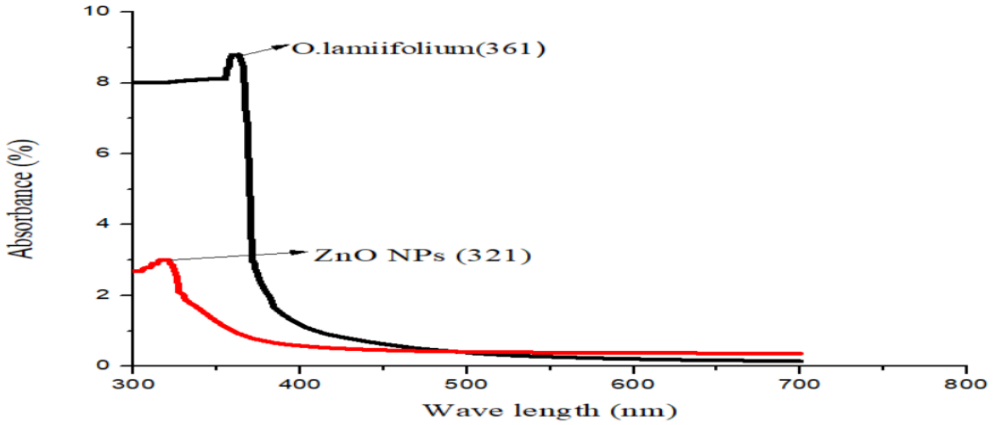


Figure 6 UV-Vis of ZnONPs (red) and O.lamiifolium leaf extract (black)

FTIR analysis

The FT- IR measurement was carried through the wave number range between 4000-500 cm^{-1} using the KBr pellet method at room temperature. FTIR spectroscopic analysis revealed the presence of different phytochemical groups, like phenolic groups, amines, ether, carboxylic acid and a hydroxyl group that acted as capping agents for the synthesis and stabilization of nanoparticles [11, 12].

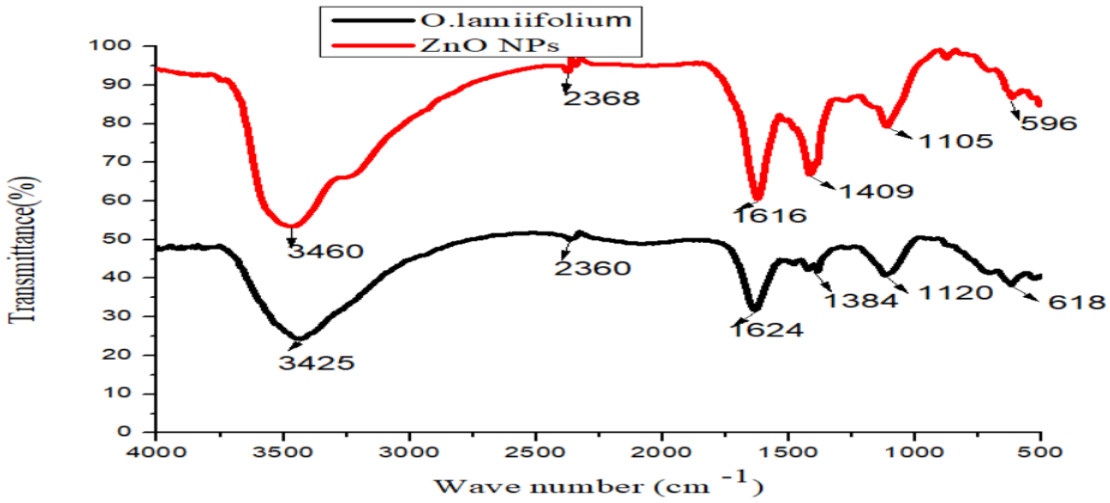


Figure 7. FTIR Spectra of bio-synthesized ZnO NPs (red) and the spectrum of O.lamiifolium (black)

The FTIR spectrum of ZnO nanoparticles has an absorption band at 596 cm^{-1}. Instinct absorption bands at 3460 and 3425 cm^{-1} appear in the presence of phenols and alcohols with free OH group [20, 21].The region of 2368 and 2360 cm^{-1} indicates the presence of symmetric stretching of COO^{-} . The absorption bands at 1616 and 1624 cm^{-1}

are characteristic of the C=C in aromatic compounds and amide I (NH).The bands at 1409 indicate C-C stretching and 1384 cm^{-1} indicate the S=O (sulfate ester) group. The band observed at 1105 cm^{-1} and 1120 correspond to the C-O group [11, 12]. The bands at 618 and cm^{-1} represent the presence of the C–Cl (alkyl halides**),** the stretch for ZnO NPs were found between 400-600 cm^{-1}. Free carboxylate groups presented in proteins can bind to the ZnO NPs and make it stable**.** Slight changes in the position of absorption bands between FTIR spectrum of the plant extract and biosynthesized ZnO NPs might confirm the role of phytochemicals in the reduction of Zn^{2+} ions and stabilization of the nanoparticles. The involvement of plant extract compounds in biosynthesizing of nanoparticles was confirmed

with a shift of peaks. Thus functional groups in the plant extract including phenolic acid and flavonoid groups play a major role in bio-reduction reaction or reducing and stabilization of NPs [1, 17].

Table 2. Summary of FT-IR analysis

Absorption	Functional group
3460 and 3425 cm^{-1}	Alcohols and phenols
2368 and 2360 cm^{-1}	Assymetric stretching of COO-
1616 and 1624 cm^{-1}	C=C aromatic and amide easter
1409 and 1384 cm^{-1}	C-C and S=O sulphate easter
1105 and 1120 cm^{-1}	C-O stretching
618 cm^{-1}	C-Cl
596 cm^{-1}	Zn-O band

XRD analysis

The XRD pattern of the synthesized ZnO NPs using O.lamiifolium leaf extract is shown in Figure 8. The XRD analysis was done to determine the crystalline nature of ZnONPs and the resulting peaks were observed at 28.8, 31.64, 34.64, 36.23, 40.83°, 45.60°,47.73,°50.92° and 56.22° which correspond to (1 0 0), (0 0 2), (1 0 1), (1 0 2), (1 1 0), (103), (200), (1 1 2) and (2 0 1) planes, respectively [13]. Hexagonal phase (wurtzite structure) of ZnONPs were compared with the standard powder diffraction card of Joint Committee on Powder Diffraction Standards (JCPDS), card No. 89–7102. The sharp and narrow diffraction peaks indicate that the product is well crystalline in nature. The average

particle size calculated by Debye-Scherer equation where full widths at half maximum (FWHM) of ZnO nano particle was found to be 22.6 nm.

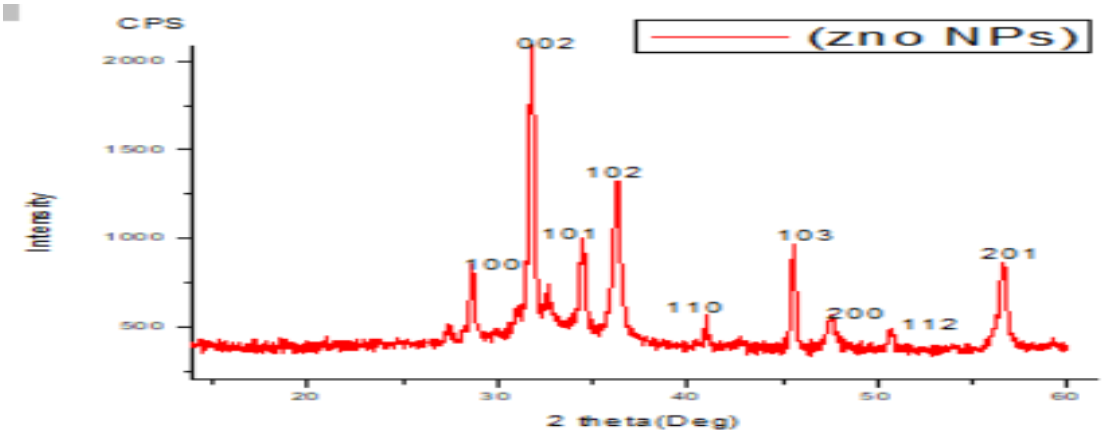


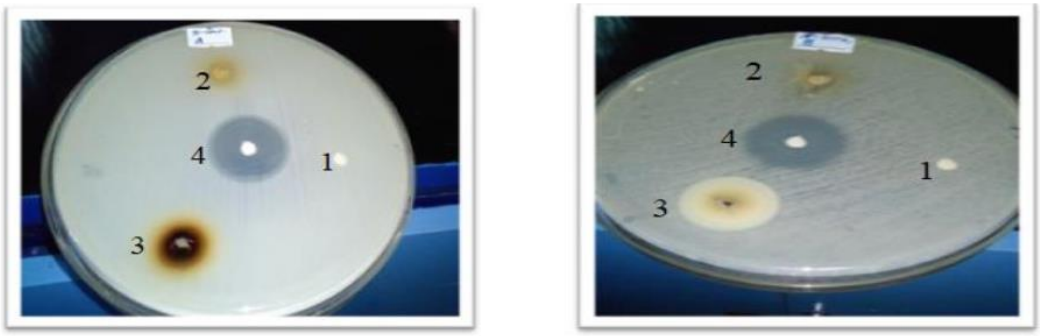
Figure 8 XRD pattern of ZnO NPs

Antibacterial Activities

The antibacterial activity of the synthesized ZnO nanoparticles was investigated against the gram-negative bacterial species (*Escherichia coli*) and gram-positive bacteria species (Staphylococcus aureus) through the disc diffusion method. The positive control (ciprofloxacin) against all the pathogens and the zone of inhibition values was measured as shown in Figure 9 and the results are presented in Table 3. The Table indicates that the synthesized ZnO NPs have shown a considerable antimicrobial activity for all the two pathogens studied; however, the highest zone of inhibition values was recorded for Escherichia coli (17.5 mm). The difference in antibacterial activity may be due to their difference in membrane structure (Gram-positive and Gram-negative). The Staphylococcus aureus (Gram-positive) bacteria have a thick peptidoglycan layer, whereas, peptidoglycan layer in the Escherichia coli, (Gram-negative) bacteria is thinner but surrounded by a lipid layer outside. In the antimicrobial activity, initially, zinc oxide nanoparticles attach to the surface of the bacterial cell membrane and then penetrate into the bacteria. After penetration, they inactivate the enzymes of the microbes, generating hydrogen peroxide. Hence bacterial cells were dead

.

(a) (b)



E.coil S.aureus

Figure 9 Antibacterial Activity of ZnO NPs Synthesized Against E.coli (a) and S.aureus bacteria (b)

Table 3. Antibacterial activity of ZnO NPs

Name of bacteria	Type	parameters			
		O.lamiifolium	ZnO NPs	Distilled H_2O	Ciprofloxacin
Staphylococcus aureus	G+ve	10 mm	16 mm	Null	25 mm
Escherichia coli	G-ve	13 mm	17.5 mm	Null	23 mm

The results revealed that gram-negative bacteria are more sensitive to ZnO NPs treatments than gram-positive bacteria, and this could be attributed to the presence of a thick layer in the cell walls (peptidoglycan) of the latter group. The bacterial activity of the synthesized ZnO nanoparticles depended also on the stability in the cultured medium [22, 23].

Conclusions

Green synthesis of ZnONPs is found to be eco-friendly and non- toxic compared to the physical and chemical methods. Ocimum Lamiifolium was one of the plants composed of many phytochemicals that were used to synthesize ZnO nanoparticles as reducing, stabilizing and capping agents for the stability of nanoparticles. The biosynthesized ZnO nanoparticles had good antibacterial activity against gram-positive and gram-negative bacteria. ZnO NPs were successfully synthesized using O.lamiifolium extract as biological material for the synthesis of nano particles. The characteristic UV- Vis absorption peak at 321 nm may have confirmed the formation of ZnONPs. FTIR analysis confirmed the presence of phytochemicals involved during the conversion of metallic ions in to nanoparticles. The crystalline nature of the

synthesized NPs was confirmed by XRD analysis. Further optimization studies should be done at different temperatures, pH, extraction concentration and time. The synthesized ZnO NPs should be characterized by other techniques like Scanning electron microscopy and transmission electron microscopy.

Aacknowledgement

The authors are grateful to the department of chemistry, Debre Markos University for accessing laboratory equipments and chemicals.

References

1. Sangeetha, G., Rajeshwari, S., & Venckatesh, R. (2011). Green synthesis of zinc oxide nanoparticles by aloe barbadensis miller leaf extract: Structure and optical properties. *Materials Research Bulletin*, *46* (12), 2560-2566.
2. Rouhi, J., Mahmud, S., Naderi, N., Ooi, C. R., & Mahmood, M. R. (2013). Physical properties of fish gelatin-based bio-nanocomposite films incorporated with ZnO nanorods. *Nanoscale research letters*, *8*, 1-6.
3. Gao, W., & Li, Z. (2009). Nanostructures of zinc oxide. *International journal of nanotechnology*, *6*(3-4), 245-257.
4. Bi, C., Li, J., Peng, L., & Zhang, J. (2017). Biofabrication of Zinc oxide nanoparticles and their in-vitro cytotoxicity towards gastric cancer (MGC803) cell. *Biomed. Res*, *28*, 2065-2069.
5. Li, M., Lv, X., Ma, X., Sun, F., Tang, L., & Wang, Z. (2007). Direct synthesis of monodispersed ZnO nanoparticles in an aqueous solution. *Materials letters*, *61*(3), 690-693.
6. Hamidi, Z. S., Abidin, Z., Ibrahim, Z., Shariff, N., & Monstein, C. (2012). Modification and Performance of Log Periodic Dipole Antenna. *International Journal of Engineering Research and Development*, *3*(3), 36-39.
7. Veerasamy, R., Xin, T. Z., Gunasagaran, S., Xiang, T. F. W., Yang, E. F. C., Jeyakumar, N., & Dhanaraj, S. A. (2011). Biosynthesis of silver nanoparticles using mangosteen leaf extract and evaluation of their antimicrobial activities. *Journal of saudi chemical society*, *15*(2), 113-120.

8. Albini, A., & Protti, S. (2016). *Paradigms in green chemistry and technology* (pp. pp-63). Heidelberg: Springer.
9. Cox, G., & Rampes, H. (2003). Adverse effects of khat: a review. *Advances in psychiatric treatment*, *9*(6), 456-463.
10. Belay, Z. (2017). *Extraction of Essential Oil from Ocimum Lamiifolium Using Steam Distillation* (Doctoral dissertation, Addis Ababa University Addis Ababa, Ethiopia).
11. Fardood, S. T., Ramazani, A., & Moradi, S. (2017). A novel green synthesis of nickel oxide nanoparticles using Arabic gum. *Chemistry Journal of Moldova*, (1), 115-118.
12. Ankamwar, B., Chaudhary, M., & Sastry, M. (2005). Gold nanotriangles biologically synthesized using tamarind leaf extract and potential application in vapor sensing. *Synthesis and Reactivity in Inorganic, Metal-Organic and Nano-Metal Chemistry*, *35*(1), 19-26.
13. Lee, J. S. (2014). Silver nanomaterials for the detection of chemical and biological targets. *Nanotechnology Reviews*, *3*(5), 499-513.
14. Patel, N. B. (2019). Khat (Catha edulis Forsk)–and now there are three. *Brain research bulletin*, *145*, 92-96.
15. Caroling, G., Vinodhini, E., Ranjitham, A. M., & Shanthi, P. (2015). Biosynthesis of copper nanoparticles using aqueous Phyllanthus embilica (Gooseberry) extract-characterisation and study of antimicrobial effects. *Int. J. Nano. Chem*, *1*(2), 53-63.
16. Zak, A. K., Abrishami, M. E., Majid, W. A., Yousefi, R., & Hosseini, S. M. (2011). Effects of annealing temperature on some structural and optical properties of ZnO nanoparticles prepared by a modified sol–gel combustion method. *Ceramics International*, *37*(1), 393-398.
17. Jyoti, K., Malik, G., Chaudhary, M., Sharma, M., Goswami, M., Katare, O. P., & Madan, J. (2020). Chitosan and phospholipid assisted topical fusidic acid drug delivery in burn wound: Strategies to conquer pharmaceutical and clinical challenges, opportunities and future panorama. *International journal of biological macromolecules*, *161*, 325-335.

18. Sahalie, N. A., Abrha, L. H., & Tolesa, L. D. (2018). Chemical composition and antimicrobial activity of leave extract of Ocimum lamiifolium (Damakese) as a treatment for urinary tract infection. *Cogent Chemistry*, *4*(1), 1440894.
19. Ruangpan, L., & Tendencia, E. (2004). *Laboratory manual of standardized methods for antimicrobial sensitivity tests for bacteria isolated from aquatic animals and environment*. Aquaculture Department, Southeast Asian Fisheries Development Center.
20. Fardood, S. T., Ramazani, A., & Moradi, S. (2017). A novel green synthesis of nickel oxide nanoparticles using Arabic gum. *Chemistry Journal of Moldova*, (1), 115-118.
21. Ankamwar, B., Chaudhary, M., & Sastry, M. (2005). Gold nanotriangles biologically synthesized using tamarind leaf extract and potential application in vapor sensing. *Synthesis and Reactivity in Inorganic, Metal-Organic and Nano-Metal Chemistry*, *35*(1), 19-26.
22. Umar, H., Kavaz, D., & Rizaner, N. (2019). Biosynthesis of zinc oxide nanoparticles using Albizia lebbeck stem bark, and evaluation of its antimicrobial, antioxidant, and cytotoxic activities on human breast cancer cell lines. *International journal of nanomedicine*, 87-100.
23. Sinha, R., Karan, R., Sinha, A., & Khare, S. K. (2011). Interaction and nanotoxic effect of ZnO and Ag nanoparticles on mesophilic and halophilic bacterial cells. *Bioresource technology*, *102*(2), 1516-1520.